

The University of Chicago

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A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE PHYSI-
CAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

1934

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E. S. FETCHER, JR.

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A METHOD FOR THE INVESTIGATION OF ELECTROSTENOLYSIS*

BY E. S. FETCHER, JR.

(From the George Herbert Jones Laboratory of the University of Chicago, Chicago)

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INTRODUCTION

The essential structural and physicochemical conditions underlying the electrical sensitivity of living protoplasm are still imperfectly understood. It is known that when an irritable tissue is traversed by an electric current, chemical reactions are set up in the tissue; these reactions furnish the free energy for the response. The general physicochemical problem has reference to the conditions under which the passage of current through a polyphasic system, consisting of a combination of aqueous solutions and non-aqueous phases in certain structural relationships, gives rise to chemical change. There are two conspicuous classes of such systems: (A) batteries or electrolytic cells, and (B) irritable living systems. Certain artificial systems of the type considered below form a third class (C). In general we find that no reaction occurs (apart from possible effects dependent on temperature) during the passage of a current through a homogeneous conductor, metallic or electrolytic. In the systems of class A, consisting in a combination of the two types of conductor, reaction does occur, but only when electric current passes between the two phases. The reaction is a boundary phenomenon; oxidation occurs where the current (positive stream) passes from the metallic phase (electrode) to the solution, and reduction where it passes in the reverse direction. For continuous electrolysis a certain critical potential (decomposition potential) is required, and a definite quantity of chemical change is associated with a definite quantity of current (Faraday's law). In living systems (class B) electrical sensitivity is bound up with a special type of structure in which electrolyte solutions containing oxidizable mate-

* This research was aided by a grant from The Rockefeller Foundation.

rials are separated from one another by thin partitions or membranes, apparently mainly non-aqueous in composition, having semi-permeability and high electrical resistance.¹ These physical properties imply high electrical polarizability, and the connection between polarization and electrical stimulation has long been recognized. A similar connection between polarization and activation exists in the system, passive iron in nitric acid (belonging in class A); here activation is a simple case of electrolysis.² In the living system also under certain conditions (brief duration of stimulating current) there is a connection between activation and the passage of a definite quantity of electricity through the irritable element.³ Living systems, however, have no metallic conductors, unless we admit the speculation that carbon chains set side by side may act as such conductors and so build up structures having the properties of electrodes. It seems more likely that the living system responds to electrical stimulation essentially because its type of structure furnishes conditions favorable to electrostenolysis or processes of a related kind. In stimulation a change in the electrical polarization of membranes is the critical event; in some way this initiates chemical reactions. The rules of polarity, of critical or threshold potential, of critical duration of flow, apply to both the living and the non-living types of electrically responsive system.² Because of the possible relation of electrostenolysis to the general problem of stimulation it has seemed desirable to make a further and as far as possible quantitative study of this phenomenon.

The phenomenon of electrostenolysis consists of the oxidation and reduction of a solution at the opposite surfaces of a high resistance membrane, which separates the two electrodes of an electrolytic cell. Reduction of the solution takes place at the surface of the membrane facing the anode, oxidation at the surface facing the cathode, and the process occurs only if there is a steep potential gradient across the membrane. Some of the effects of the membrane are the same as those of a metallic conductor in the same position, in so far as ions are oxidized and reduced at the surfaces of both. The definition

¹ Cf. Blinks, L. R., *J. Gen. Physiol.*, 1929-30, **13**, 361, 495. Osterhout, W. J. V., *Biol. Rev.*, 1931, **6**, 369 (pp. 397 ff.).

² Lillie, R. S., *J. Gen. Physiol.*, 1935-36, **19**, 109.

³ Cf. Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1936, **119**, 305 (cf. p. 317).

stated above is more general than any so far given. Hitherto most workers have been interested in the formation of metallic deposits on the membrane surface, and have defined the phenomenon in terms of these. However, as will be shown, these deposits are not its only manifestations, and the above broader definition is needed.

Although many workers have experimented with this phenomenon, hitherto no one has applied quantitative methods to it;⁴ consequently such data as exist are of relatively little value. The present investigation was undertaken in order to obtain quantitative information on the efficiency of the process under varying conditions. Because of the very small amounts of material transformed, the difficulty of obtaining satisfactory results has been great, hence so far few data have been obtained. However, a method has been developed which, on further application, promises to give reliable quantitative results.

EXPERIMENTAL

The first experimental requirement for a quantitative study of this phenomenon is that the oxidation or reduction to be measured in the solution should take place only at the membrane surface. This means that the experiments must be performed in a closed system in the absence of oxygen and hydrogen; that the solution be free from any other impurities which would tend to oxidize or reduce it; and finally that the electrodes of the cell be not too near the membrane and that diffusion of solution from their neighborhood be minimized. Secondly, the membrane must be inert in the sense that it must not oxidize or reduce the solution without the passage of current. It should also be electrically stable; *i.e.*, its resistance should not vary excessively during the course of an experiment. Further, it should not entrap products of the stenolysis, which means that the membrane must be thin. Since the potential gradient across it must be comparatively high to produce any oxidation or reduction, the conductivity of the material used for making the membrane should be low, with as high a break-down potential as possible. Other general requirements for a suitable apparatus are that there should be, adjacent to the membrane, a small volume of solution, separable from the remainder of the solution in the cell, which it is possible to

⁴ There is a summary and bibliography in Freundlich, H., *Colloid and capillary chemistry*, New York, E. P. Dutton, 3rd edition, 1926, 271. Also see Grotthuss, T.v., *Ann. Phys. u. Chem.*, 1819, **61**, 65. Recent papers which emphasize especially the polar nature of the phenomenon are: Lillie, R. S., and Pond, S. E., *Am. J. Physiol.*, 1922, **63**, 415; Guastalla, L., and Urbain, G., *Compt. rend. Acad.*, 1934, **198**, 1679; 1935, **201**, 268. For a recent theoretical discussion *cf.* Söllner, K., *Z. Elektrochem.*, 1929, **35**, 789.

analyze. Provision must also be made for the easy and positive installation of the membrane in such a manner that, when in place, all of the current shall pass through, and none leak around it.

The use of a potentiometric method of analysis was, from previous experience with colorimetric analysis, judged to be the best method for a determination of the amount of material transformed at the membrane. If, for example, in the ferrous-ferric oxidation, before and after a known quantity of current is passed, the potentials of the solution are measured against an inert platinum electrode, the change in ratio of reduced to oxidized ions can be calculated from the equation:

$$E_h = E_0 - \frac{RT}{nF} \ln \frac{(\text{Fe}^{++})}{(\text{Fe}^{+++})}.$$

If the volume and concentration of the solution are known, the number of moles of material so changed per unit current may be computed. The solution to be used as an indicator must be in a reduced state for two reasons: first, to make sure of the absence of oxygen, and secondly, to take advantage of the slope of the E_h vs. (Reductant)/(Oxidant) curve, so that a small change in ratio may produce a large change in potential.⁵

The final form of the apparatus is shown in Fig. 1. This cell and the earlier cells were designed with a view to the investigation of compounds which would form no deposit on the membrane. The chamber B in this cell is equipped with three platinum wire electrodes, sealed into soft glass tubes; and contains also the terminus of a saturated calomel cell, connected to it by a ground joint and stop-cock, which can be filled with oxygen-free potassium chloride solution from the reservoir. One of the platinum electrodes is used, in conjunction with a similar one on the other side of the plate A, to measure the voltage drop across the membrane which is fastened on this plate. The drop of potential in the centimeter of solution which separates them is negligible in comparison. The solution in B is kept stirred, while the potential measurements are in progress, by moist nitrogen bubbled into it through a tube at the base of the chamber, attached to the nitrogen supply and pump line. The principal connection of this line is through the left-hand electrode, or cathode, chamber. The glass springs make allowance for slight differences in the position of the cell when it is set up; they allow the connections to be readily resealed, and the ground joint of the calomel cell to be put together with very little strain. The "square helix" used was found to be far more flexible than an ordinary helix. The cell is tightly clamped at the cathode chamber only and loosely supported elsewhere. If otherwise mounted, it was found that it was impossible to eliminate strains sufficiently to prevent its cracking. The large stop-cock, the core of which is shown enlarged (c), combines several simple cocks, thus facilitating the manipulation of the

⁵ See Clark, W. M., Studies on oxidation-reduction, I-X, *Bull. Hyg. Lab., U. S. P. H. S.*, No. 151, 1928.

solution. The reducing flask is two flasks in one: the outer contains platinized asbestos and in this the reduction takes place; the inner one, provided with a linen filter at the bottom, serves to store the solution out of contact with the platinized

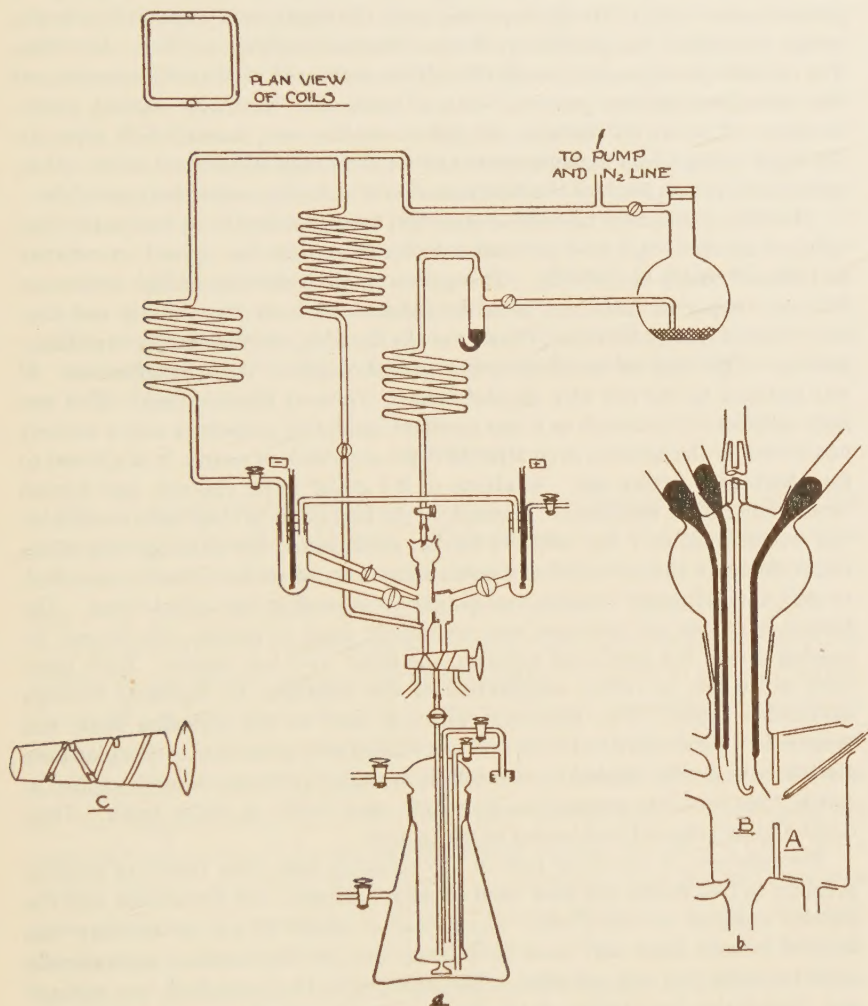


FIG. 1

asbestos. To be able to increase the hydrogen pressure in the outer flask without blowing apart the joint, it is sealed with Piccin cement. The flask is supported in position on a jack and is connected to the nitrogen and hydrogen lines with

flexible copper tubing (Bendix Aviation Corporation) sealed on to the glass tubes with de Khotinsky cement. This tubing was used in preference to rubber because of the noticeable diffusion of oxygen through the latter.

For the measurement of the voltage drop across the membrane, a 40 volt potentiometer with 1/10 volt steps was used; the insertion of 80,000 ohms in the circuit minimized the possibility of error from electrolysis at these electrodes. The current which passed through the cell was measured with a milliammeter, and the oxidation-reduction potential with a Leeds and Northrup student potentiometer. This circuit contains two galvanometers; one, a small desk type, for the rapid voltage drop measurements and for the rough adjustment of the other, more sensitive one, used for the determination of oxidation-reduction potentials.

The most satisfactory membrane of several tried appeared to be that made from cellulose acetate. A 1 to 3 per cent solution in acetone was spread on mercury and allowed to dry very slowly. This gave a stable membrane of high resistance. Its mean apparent thickness could be determined from the volume and concentration of the solution used, the area of the dry film, and the density of cellulose acetate. This type of membrane was used throughout these experiments. It was fastened to the cell with glyptal cement (General Electric Co.). This was very satisfactory inasmuch as it has excellent insulating properties and is entirely unaffected by the solution even after 10 days; provided, of course, it is allowed to dry thoroughly before use. Solutions of 0.1 molal ferric chloride and ferrous ammonium sulfate were used. A sample of the best grade of these salts obtainable was weighed out to 1 mg. without further purification, for although impurities might influence the potential measured, this error would be virtually cancelled, as only the difference between two potentials is used in the calculations. The tanked hydrogen and nitrogen were effectively freed of oxygen, the former by passing it over hot platinized asbestos, the latter over hot copper. Both gases were moistened, to reduce evaporation of the solutions, by bubbling through oxygen-free water. The platinized asbestos used in the reducing flask was prepared by the ordinary method, and was washed with great care. The platinum electrodes in B were cleaned by electrolysis in strong hydrochloric acid, washed in water, then in dilute ammonium hydroxide, and finally in water again. They could also be removed and heated to white heat.

The solution, in the outer part of the reducing flask, was boiled at reduced pressure to free it and the flask from oxygen; hydrogen was introduced and the process repeated several times. A pressure of about 15 cm. of mercury was applied to both inner and outer containers, and the flask shaken mechanically until the reduction was complete. The pressure on the inner flask was released and the solution forced through the filter up into it, enough pressure being maintained in the outer flask to support the solution therein. Nitrogen was then passed through it for 3 or 4 hours to remove dissolved hydrogen. With the reducing flask in place under the cell, the system was freed of oxygen by alternately evacuating with an oil pump and filling it with pure nitrogen. The cell

was then filled with solution and allowed to stand overnight to soak the membrane and to be certain of the absence of oxygen. (Its presence could be detected by a change of the potential of the solution.) Saturated potassium chloride was run into the end of the calomel electrode, and while the solution was stirred, measurements of its oxidation-reduction potential were made until the readings became constant. After the stop-cocks were properly adjusted, current was passed between the two platinum coils in the electrode vessels, its direction being such that oxidation took place in B. Readings were made, as nearly simultaneously as possible, of the current, the voltage drop across the membrane, the time, and the temperature. Cotton plugs inserted in the bores of the two large stop-cocks which lead to the electrode vessels, and which were necessarily open during the passage of the current, reduced diffusion from these vessels to the vicinity of the membrane. After a known interval of time, the current was shut off, these same two stop-cocks closed, the cock in the vent above B opened, and the solution therein stirred. The oxidation-reduction potential was then measured until the readings became constant; the constant value serving for the final reading of the first experiment and the initial reading for the second, which was carried out immediately.

RESULTS

Fig. 2 shows the results of some rather inaccurate colorimetric experiments on methylene blue. The efficiency of the process in terms of equivalents oxidized per faraday passed, is plotted against the voltage drop per centimeter across the membrane. Each symbol corresponds to a series of experiments. The broken line was drawn without regard to the points designated as belonging to the 3-21 or 3-27 series. It would not be wise to attempt to draw definite conclusions from these data because of their uncertainty. However, there does seem to be a minimum potential gradient necessary to oxidize the dye. This is in the neighborhood of 6700 volts per centimeter. It will also be noticed that most of the above mentioned points (of the 3-21 and 3-27 series) show a relatively high efficiency; in these runs ferrous iron was present in small quantities. If this greater efficiency is real, the iron seems to act as a catalyst for the oxidation. This is of special interest in view of the rôle played by iron as oxidation catalyst in living tissues where it (or other similar elements) is invariably found. The most important and most certain conclusion which may be drawn is that methylene blue is subject to stenolytic decomposition. No organic molecule has previously been shown to be affected by this process.

It was found that not only was the leuco-methylene blue oxidized, but that the insoluble methylene white base was often deposited on the surface of the membrane facing the anode.

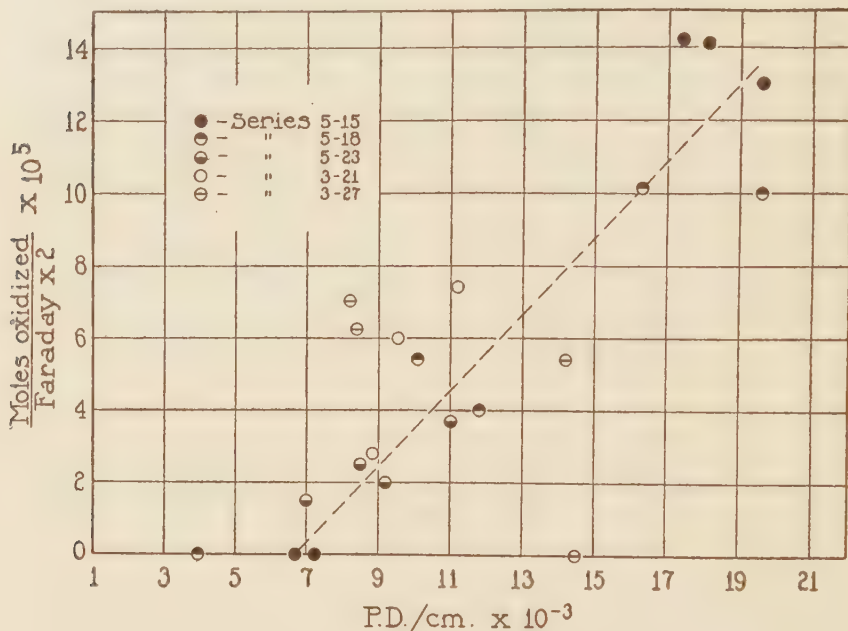


FIG. 2

The results obtained by the use of the potentiometric method are all for the ferrous-feric system. The number of moles of ferrous ion oxidized is given by the equation:

$$E_h = E_0 - \frac{RT}{nF} \ln \frac{(\text{Fe}^{++})}{(\text{Fe}^{+++})}$$

E_h is the potential measured referred to the hydrogen electrode. (The voltage of the calomel cell used was taken as +0.2458 at 25°C.) E_0 is the normal oxidation-reduction potential, taken as +0.7434 at 25°C.⁶ R is the gas constant, T the absolute temperature, n the number of electrons involved in the change from one state to the other,

⁶ Popoff, S., and Kunz, A. H., *J. Am. Chem. Soc.*, 1929, **51**, 382.

and F is the faraday constant. The number of moles of ferric ion present can be calculated by the following relation from the ratio (r) of the electrode equation, if the volume in cubic centimeters (v) of the solution in the chamber and its molal concentration (M) are known:

$$\frac{m}{r + 1} = \text{moles Fe}^{+++}, \text{ where } m = \frac{vM}{1000}$$

Since E_h was measured before and after the passage of the current through the membrane, the initial and final number of moles of ferric ion could be calculated. The difference between these quantities is the number of moles of ferrous ions oxidized to ferric. The area under the time-current curve was measured in order to evaluate the number of coulombs passed. The ratio of the number of moles of Fe^{++} oxidized, to this quantity, gives the efficiency. The potential drop across the membrane was measured directly; the mean apparent thickness of the latter was calculated from its area, the volume and concentration of the solution used to make it, and the density of cellulose acetate,—1.26 gm./cc. The data of a typical experiment are given in Table I; t is the time; I , the current in milliamperes; P.D. is the potential drop across the membrane in volts; E_1 and E_2 are the potentials of the two electrodes used to measure the oxidation; T is the temperature in degrees Centigrade. The solution used was 0.1 M FeCl_2 .

In Table II the data obtained by this method are summarized, arranged in order of increasing voltage drop across the membrane. The majority of the experiments are not represented in the table; for as many as fifteen were made in one series. When the results were examined, it was found that the later experiments of each series seemed to be far more efficient than the earlier ones, irrespective of the potential across the membrane. After the first few experiments the effect increased with the time elapsed after the series was started, and with the total current passed during the series. These latter quantities are closely related. The curve was flat for the first few hours, then rose very steeply, and finally started to level off. A rough calculation showed that it would take about 8 hours for a ferric ion to be carried from the anode to the membrane; this time is decreased by the agitation of solution caused by the turning of stop-cocks, diffusion,

etc. Apparently then, transference of the ferric ion formed at the anode through the membrane into the chamber where the analysis was

TABLE I
Experiment Q₁ of Table II. Solution 0.1 M FeCl₂

<i>t</i>	<i>I</i>	P.D.	<i>E</i> ₁	<i>E</i> ₂	<i>T</i>
8:33	—	—	+0.2402	+0.2402	23.8
8:41	—	—	0.2402	0.2402	23.7
8:50	—	—	0.2402	0.2402	23.9
8:57	—	—	+0.2402	+0.2402	23.9
9:00	17.6	1.7	—	—	—
9:03	17.3	2.4	—	—	24.0
9:05	17.15	2.7	—	—	—
9:07	17.1	2.8	—	—	—
9:10	17.05	2.8	—	—	24.0
9:15	17.0	2.8	—	—	—
9:20	17.0	2.7	—	—	24.0
9:25	17.0	2.7	—	—	—
9:30	16.9	2.7	—	—	24.1
9:35	16.8	2.7	—	—	—
9:40	16.8	2.7	—	—	24.0
9:45	16.8	2.7	—	—	—
9:50	16.8	2.7	—	—	24.1
9:55	16.8	2.7	—	—	—
10:00	16.75	2.7	—	—	—
10:04	—	—	+0.2443	+0.2440	24.2
10:10	—	—	0.2440	0.2438	24.2
10:15	—	—	0.2437	0.2436	24.1
10:21	—	—	0.2436	0.2435	24.1
10:30	—	—	0.2435	0.2435	24.1
10:40	—	—	+0.2435	+0.2435	24.2

Average potential drop..... 2.70 volts

Thickness of membrane..... 5.33×10^{-4} cm.

Potential drop/cm..... 5.07×10^3 v./cm.

No. moles oxidized..... 1.30×10^{-8}

No. faradays passed..... 6.34×10^{-4}

No. moles oxidized/faraday..... 2.05×10^{-5}

made was the cause of the anomalously great efficiency observed after the first experiments. After a time the ferric ions should enter the

chamber B through the membrane at the same rate they leave it; this approach to equilibrium is observed in the "efficiency"-time curve referred to above. On the basis of this reasoning, all runs after the first three were discarded. At the start of a series, transference across the membrane could not cause important errors because the solution on both sides was the same. The effect (in the opposite direction) of diffusion through the membrane was likewise minimized by the selection of only the first runs, and the rejection of runs in which the potential did not become rapidly constant. Runs were also discarded when the potentials of the two oxidation-reduction electrodes differed by

TABLE II

Experiment No.	No. faradays	No. moles oxidized	Moles oxidized No. faradays	P.D./cm. $\times 10^{-3}$	P.D. (Obs.)
P-1	4.44×10^{-4}	0	0	1.27	11.9
P-2	4.92	0	0	1.45	13.2
T-2	1.99	4.3×10^{-9}	2.16×10^{-5}	1.83	2.65
T-3	2.83	4.1	1.45	2.21	3.20
P-3	6.42	0	0	2.32	17.2
T-1	3.87	6.6	1.71	3.24	4.70
Q-1	6.34	13.0	2.05	5.07	2.70
Q-3	8.29	21.0	2.5	7.88	4.20
Q-2	8.44	15.0	1.8	8.08	4.30
U-4	2.24	3.0	2.3	13.3	19.3
U-3	1.36	3.0	2.2	15.8	22.7
U-2	1.64	4.0	2.4	16.4	23.8

more than 0.1 mv.; usually it was not possible to read any difference between them on the student potentiometer. The greatest accuracy is realized only when the order of magnitude of the number of moles of ferrous ion oxidized is the same as the number of moles of ferric ion present. If this condition is not fulfilled, one, or even two decimal places will be lost when the difference is taken between the initial and final values of the latter. Another error is caused by the too large volume (20 cc.) of the chamber where the analysis is made; however, if this is reduced too far, the errors of diffusion and transference become much more important. The errors relative to the determination of the number of faradays, the average potential drop,

and the thickness of the membrane are negligible compared to those mentioned above.

The data of Table II are plotted in Fig. 3 which gives the efficiency of the process in terms of moles oxidized per faraday passed, against the potential drop per centimeter across the membrane. It seems from the shape of the curve that there is a minimum potential gradient below which no oxidation occurs; this minimum falls at about 2150 v./cm. Lillie and Pond⁴ estimated 2000 v./cm. as the minimum at which they could effect any oxidation of ferrous ion at the surface of a rubber membrane. Considering the inaccuracies involved in the determination of the thickness of the membrane, this is excellent agreement. Whether the actual curve follows in detail the one in

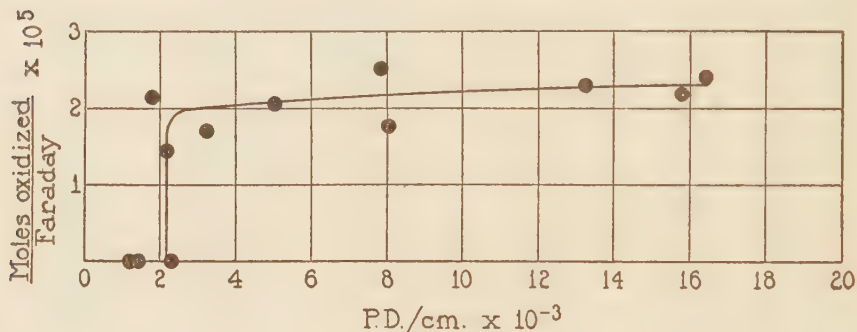


FIG. 3

the figure must be determined by further experiment, for not enough data are available to make this curve certain in every respect. Although no data are presented, and those available are of doubtful accuracy, there is some evidence that the efficiency begins to fall off above about 17,000 v./cm. possibly falling again to zero in the neighborhood of 22,000 v./cm.

Any extensive theoretical treatment of this subject, based on data at present available, must necessarily belong largely in the realm of speculation. Nevertheless, a few suggestions of possible mechanisms concerned in this reaction seem not inappropriate. First, the possibility that electrostenolysis is due to capillary-electrical fields, as Coehn⁷ suggested, seems remote, inasmuch as these fields become

⁷ Coehn, A., *Z. Elektrochem.*, 1898, **4**, 501; *Z. physik. Chem.*, 1898, **25**, 651.

negligible at the salt concentrations used. In this connection it would be interesting to investigate the change in the effect with the concentration. It is not beyond the range of possibility that an induced charge on the membrane, acting in conjunction with the relatively high velocity of the ions (approximately 1.3 cm./sec. at 2000 v./cm. for ferrous ion) which pass through the membrane pores, would serve to discharge some of them; by some such hypothesis, the minimum potential could be explained, and, if it exists, probably also the maximum potential. A study of the change of the efficiency and of the possibly more significant quantity, the minimum stenolytic potential, would show whether the membrane acts in some special capacity, or whether it merely serves to concentrate the ionic flow into several minute pores in which the stenolysis is effected. On the basis of the agreement between the minimum stenolytic potential found in this investigation and that found by Lillie and Pond with a very different membrane, although this agreement may be purely coincidental, the latter hypothesis seems the better. If the minimum stenolytic potential found for methylene blue (6700 v./cm.) is approximately correct, the difference between this value and that for iron indicates a variation of this quantity with the ion involved; most probably a connection not only between the size of the ion, but also its normal oxidation-reduction potential.

No purpose could be served by further speculation, since the main object of this paper has been to present a method, with sufficient data secured by its use to demonstrate its feasibility.

SUMMARY

A quantitative method for the investigation of electrostenolysis has been developed. Electrostenolysis is redefined in the light of the discovery that organic molecules are subject to it. The experimental requirements for a quantitative study are enumerated, and the apparatus and procedure described.

It is found that with ferrous and ferric ions and a cellulose acetate membrane, the potential drop across the membrane must be above about 2150 v./cm. in order to effect any oxidation and reduction. With the present apparatus and conditions the ratio of equivalents oxidized or reduced to faradays passing the membrane is low,—of the order of one to several thousand.

